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Prospective Sciences: Computational Bioanalytics

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Computational methods allow 'prospective sciences'. Statements like 'let's see how this would look like' or 'does this match current knowledge' drive *in silico* investigations even in fields of architecture and of course the natural sciences. In the latter it supports materials sciences as well as the drug discovery process. There is almost no field in which the computer is not 'visualizing' the ideas and tasks of the experimentalists. Here, the focus is on the application of computational bioanalytics for structural modeling of viral channel forming proteins in order to give an example.

Viruses encode this special type of protein, which is anchored in lipid membranes. The membranes could be either found in envelope viruses or in subcellular compartments of an invaded host cells. These proteins are mostly around 100 amino acids in length and are produced as monomeric units. In a consequent step, they need to assemble to form ion and/or substrate conducting pores. With these proteins the virus alters proton and electrochemical gradients across the lipid membrane which leads to conformational changes of other viral proteins or alters the chemical environment in cellular sub-compartments [1,2].

To mention just three of the representatives of this class of proteins: M2 from influenza A virus. This protein forms tetramers and conducts protons. It helps to initiate the entry of the virus into the cell by allowing the protons to enter the virion and alter the conformation of the fusion protein haemagglutinin. In the late stage of the infectivity cycle, M2 maintains a low pH in sub-compartments so that the fusion protein is produced in its non-fusion-active form. M2 is very well characterized by experimental methods such as NMR spectroscopy and X-ray crystallography. It has been the first target protein in antiviral therapy. Amantadine and derivatives have been used to treat the flu.

Protein p7 from Hepatitis C virus is another hot target in antiviral therapy. The polytopic protein has two transmembrane domains (TMDs), which is one more than in the bitopic M2. This monomer is assembled in either hexameric or heptameric bundles which are supposed to be ion conducting. Currently, electron microscopic data of the assembled protein are available. Also NMR spectroscopic investigations have been done, which give structural data about the monomer. Against this protein a highly potential drug has been found, BIT225. And if this protein cannot work for the virus, the virus cannot replicate!

Another representative is Vpu from HIV-1. Reported to be a bitopic protein forming channel, it is also known to interact with host proteins, leading to their down-regulation by the cellular proteasomal machinery. Structural information is available from solid state and solution NMR spectroscopy. Again, these data are only used for the interpretation ofa 'monomer'. It seems that BIT225 is a good drug candidate against Vpu. Vpu is called an auxiliary protein, since other HI viruses, such as HIV-2, do not have this protein. HIV-1 can survive without Vpu, although to a much lesser extent. So it seems that Vpu is also a sensible target to combat HIV-1.

Despite investigations for more than 30 years for some of these proteins, there are a lot of important issues which are yet to be discovered. For none of them there is afull length structure known whilst assembled forming a pore. For some of them we even do not know how many monomers form the pore (e.g. Vpu). For others, which have not yet been mentioned, we do not have any experimental information about the structure at all (e.g. 2B from Polio and Coxsackie viruses, E5 from human papilloma virus, 3a and 8a [3] from SARS-CoV). How about drugs? Some poses have been experimentally characterized (e.g. M2) and others have been proposed by molecular biological methods (e.g. p7, E5). Structure based visualization of the latter have be achieved using computational modeling.

What can computational bioanalytics do? Because of the low dielectric hydrophobic environment of the lipid membrane it is possible to 'suggest' a transmembrane stretch in a protein and assembly motifs [4,5]. Obviously, hydrophobic amino acids should be used to span the bilayer. Usually the accumulation of about 20 – 25 hydrophobic amino acids within almost the same overall number of amino acids identifies such atransmembrane stretch or domain. Using bioinformatics tools this stretch can be identified. It is even possible to identify this stretch as a helix based on the type of amino acids. Experimentally it is confirmed that the overwhelming majority of the membrane proteins use a helical motif rather than a β-sheet motif to cross the lipid membrane.

In a next step, the amino acid sequence can be taken and modeled into an ideal helix simply by using software tools [6-8]. The software generates the helices according to generally accepted φ and ψ angles found for the amide bonds in experimental structures in which helical motifs are present. From this step onwards, there is some 'freedom' to carry on. After copying the generated helix into as many copies as needed, the 'assembly protocol' can be started to 'form' the bundle. Of course, at this stage, it is possible to use the experimentally derived monomeric structures to form the pore as well. With the biology in mind it is anticipated that the monomers can diffuse within the lipid bilayer in order to meet each other. During that time the monomer should adjust to the environment of the lipid bilayer. This stage of the assembly process can be mimicked using special types of molecular dynamics (MD) simulations covering various size and time scales. After 'treating' the protein with the lipid membrane, it can be used again in a docking approach to generate the bundle. Screening for conformers with low energies there are various options to do the assembly mechanically, either (i) docking all monomers simultaneously or (ii) built up the oligomer in a step-wise fashion [3]. With protocols like this,

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some of the current ideas of how the bundle structure should look like have been given for the first time (e.g. Vpu, p7 [9], E5, 2B [10], 3a [7]). It needs to be stated that the protocols mentioned are based on force field based scoring functions. It is obvious that the protocol described has also its limitations. Obviously, when a protein has too many TMDs the results harbor too many ambiguities. In a final step the generated models would need to undergo a quality check. Consequently methods to calculate e.g. the potential of mean force (PMF) of ions traversing the putative pore would give information about conductance and selectivity. If a structure has passed all thesesteps it could be used for drug docking.

It is definitively a desire in bioanalyticsto improve the protocol of membrane protein assembly. The protocol should also include further protein folding ideas [11], especially when the extramembrane parts of the protein have to be modeled in the future. In the case of Vpu, it has been found that parts of the cytoplasmic domain interact with a host protein. It needs to be investigated whether other channel proteins do also have extramembrane binding sites which on the other hand could be important drug binding sites. Improving calculations for conductivity is important for quality assessment of the generated models.

Improvements in the field of computational bioanalytics go parallel with developments in computational biophysics. Similarly to experimental methods, the computational methods need to be validated against experiments. While using these methods to hunt for the 'unknown' or predict the 'future' it is important to know about their quality and limitations.Computational methods always 'propose', never 'verify'.

The protocol described can be seen as a 'short cut' to structure. But would it not be more appropriate to wait for the experimental structure to be solved? In most of the drug discovery fields many drugs have anyway been developed without the knowledge of the target structure. However, as in the field of antiretroviral therapy or antibiotics, the speed with which organisms develop resistance is stunning and will not allow for a 'let's wait foramoment' attitude. Consequently computational tools will support a fast analysis of structure as well as protein ligand interactions. This will reduce not only time in drug development but also in the development of novel materials.

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